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EFFECTS ON TOXICITY OF VOLATILE PRIORITY POLLUTANTS ADDED TO A CONVENTIONAL WASTEWATER TREATMENT SYSTEM

by

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16. ABSTRACT

Static acute, unaerated, toxicity tests using fathead minnows and Daphnia magna and a bacterial toxicity assay, Microtox™, were conducted on samples of influent and effluent from two conventional activated sludge pilot wastewater treatment systems. The two pilot treatment systems (A and B) were constructed and operated in an identical manner except that a mixture of 16 volatile priority pollutants was continuously added to the influent of the experimental, B system. The common, unspiked influent for both systems was a mixed industrial and domestic wastewater. The volatile priority pollutants were added to system B to obtain a nominal concentration of 50 ug/1 each. The toxicity tests were performed on the influent, primary effluent, and secondary effluent samples to determine the acute toxicity of the various samples and to compare the reduction in toxicity across the two treatment systems. The results of these tests indicated that there was no difference in toxicity reduction between the two pilot treatment systems at the level of pollutants added. Toxicity for pairs of similar samples, influent A and B, primary effluent A and B, and secondary effluent A and B, was essentially the same. Even the influent samples, where the highest concentration of pollutants would be expected in the B samples, were not different.

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Introduction

The Federal Water Pollution Control Act of 1972, P.L. 92-500 and the Clean Water Act of 1977, P.L. 95-217 require the U.S. Environmental Protection Agency to identify toxic materials discharged into the surface waters of the United States and to promulgate regulations for control of such discharges. Further, the Consent Decree (National Resource Defense Council, et al, vs. Train, 1976) specifically identifies 129 compounds, known as the "priority pollutants", for which regulations are to be promulgated.

To promulgate regulations limiting the discharge of "priority pollutants" and toxics, information is required on how well toxic pollutants are treated or removed in waste treatment facilities, how the pollutants affect the treatment systems, and where the pollutants are distributed and concentrated or released in the treatment systems.

As part of a project by the Municipal Environmental Research Laboratory - Cincinnati (MERL) to evaluate the behavior and fate of volatile priority pollutants in conventional, municipal wastewater treatment systems, aquatic toxicity tests were conducted by staff of the Environmental Research Laboratory - Duluth/Newtown (ERL-D/N). The primary objective of the toxicity testing was to biologically determine toxicity and toxicity removal across conventional treatment systems. The biological data were then to be used to supplement MERL's physical and chemical evaluation of the treatment systems. The volatile organic priority pollutant study was one of a series of MERL projects designed to determine the capacity of conventional wastewater treatment systems to treat "priority pollutants".

Static acute toxicity tests using fathead minnows, Pimephales promelas, and an invertebrate, Daphnia magna, and a bacterial toxicity assay, Microtox™

(Beckman Instruments, Inc., Microbics Operations, Carlsbad, California) were conducted on the influents and effluents from two conventional activated sludge pilot treatment systems. The treatment systems were identical except that a mixture of 16 volatile organic priority pollutants was continuously added to one of the systems. The pilot treatment systems were designed, constructed and operated by MERL at the U.S. Environmental Protection Agency's Test and Evaluation, (T&E Facility), Cincinnati, Ohio.

Materials and Methods

Pilot treatment systems. The treatment systems consisted of two 133 l/min. conventional, plug flow, activated sludge systems. A schematic diagram of the systems is given in Figure 1. and the operating characteristics of the systems are given in Table 1. The control system (A) received a mixed domestic and industrial waste influent. The experimental system (B) received the same influent as A except a mixture of 16 volatile priority pollutants dissolved in methanol was continuously added to give a nominal concentration of 50 µg/l each in the influent (Table 2.). A concentration of 50 µg/l each was chosen because it was measurable and at the high end of concentrations of the pollutants typically found in municipal treatment plant influents. The detailed description of the operation of the pilot system and the methods for chemical evaluation are given in Petrasek[1].

Sampling and sample handling. Grab samples for toxicity tests were collected from sampling ports on the treatment systems. Primary and secondary effluent sampling was scheduled, based on calculated and measured detention times of the treatment systems. In that way, the primary and secondary effluent samples were taken from the same plug of waste water from which the influent sample was taken.

All samples were collected in stainless steel containers. Toxicity tests were begun on the samples within two hours of collection. Samples for the fish and Daphnia tests were not treated or modified except for a temperature adjustment. Samples for Microtox™ tests were adjusted for both temperature and salinity.

Dilution water. Dilution water for the fathead minnow and Daphnia tests, as well as for culture and holding, was a mixture of dechlorinated, deionized Cincinnati tap water and Newtown Laboratory spring water made to an approximate hardness of 200 mg/l (as $CaCO_3$). The water was made up at the ERL-Duluth/Newtown Laboratory and transported to the T&E facility. At the T&E facility, the water was held at $23 \pm 3^{o}C$ and aerated in a covered 2000 liter fiberglass storage tank until used. Dilution water for the microtox assay was Microtox™ Reagent Diluent from Beckman Instruments, Inc. Prior to use, the diluent was stored at $2^{o}C$.

Test organisms. Fathead minnows were obtained from a culture unit at the ERL-Duluth/Newtown laboratory and Daphnia magna were from a culture maintained at the T&E facility. Luminescent bacteria, Photobacterium phosphoreum, Microtox™ Reagent, were obtained from Beckman Instruments, Inc. The fathead minnows were transported to the T&E facility three days before being tested. They were held at the T&E facility in a static renewal system in which 90% of the holding water was replaced once every 24 hours. Culture temperatures and holding and acclimation temperatures were maintained at $23 \pm 3^{o}C$ for both fish and Daphnia. Prior to use the Microtox™ Reagent, bacteria, was refrigerated at $2^{o}C$.

Fish were not fed for 48 hours before use. Daphnia, however, were fed until placed in test containers. The fish used for testing were from 18 to 42 mm in length and 0.08 to 0.32 gm. The Daphnia were first instars.

Toxicity tests. The fish and Daphnia static acute toxicity tests were conducted using the basic guidelines outlined by Peltier[2]. The choice of alternative, static, unaerated procedures were dictated by conditions unique to the

study. Microtox™ toxicity assays were conducted according to an assay procedure with duplicate determinations, Beckman Instruments, Inc.[3].

The toxicity tests were conducted in two series. In the first series, only influent and secondary effluent samples were tested for toxicity with fathead minnows and Daphnia. In the second series influents, primary effluents, and secondary effluents were tested with fathead minnow, Daphnia, and Microtox™. Test solution volumes for the two series of tests were, respectively, 16 and 8 liters for the fathead minnows and 200 and 100 ml for the Daphnia tests. Test containers for the fish test were 19.6 liter wide mouth glass jars. Test containers for the Daphnia for the two series of test were, respectively, 250 and 150 ml glass beakers. Ten fish were used per test concentration and control in both series without replication. Eighteen Daphnia were used per test concentration and control, 6 per replicate with three replicates. Duplicate test concentrations and controls were run for the Microtox™ assay as described in the operations manual. Test temperatures were nominally 23 $\pm$ 3°C for the fish and the Daphnia tests and 15°C for the Microtox™ assay. Fish test solutions were volume to volume, proportional dilutions of sample with diluent water. Test solutions for the Daphnia were made by taking aliquots of the fish test solutions. For the fish and the Daphnia, six test concentrations and a control were used. For Microtox™, four test concentrations and a control were set up using a serial dilution procedure. Each test concentration for the fish and Daphnia tests and the Microtox™ assay was 0.5 of the next higher concentration. Fifty percent was usually the high influent and primary effluent test concentration and 100% was the high secondary effluent concentration for the fish and Daphnia tests and Microtox™ assay. In the second series of tests, only 100% or 100 and 50% concentrations and a control were usually set up for the secondary effluent samples.

Test duration for the fish, Daphnia, and Microtox was, respectively, 96 hours, 48 hours, and 15 minutes.

Chemical and physical measurements. A multiparameter U-7, Water Quality Checker (Horiba Instrument Corporation, Irvine, California.) was used to measure dissolved oxygen, pH and temperature initially and every 24 hours during the fish test in all concentrations. The same measurements were made for the Daphnia at the end of the 48 hour test period. The initial fish test measurements were also used as the initial Daphnia measurements since the Daphnia test solutions were aliquots of the fish test solutions. No measurements were made on the Microtox test concentrations because of the small volume, 1 ml. Alkalinity and hardness measurements were also made on the high, medium, and low fish test concentrations and control water at the beginning of each test using American Public Health Association, et al[4] procedures.

Data analysis. Ninety-six hour LC50 and 48 hour EC50 values with 95% confidence limits for the fathead minnow and Daphnia tests were calculated using a computer-adapted, moving average-angle procedure of Harris[5]. Microtox™ 15 min-EC50 values without confidence limits were calculated using the gama decrease method in the Microtox™ Manual. Fish and Daphnia LC50 and EC50 values were considered different when their 95% confidence limits did not overlap. Microtox values were considered different if their EC50 values differed by a factor of two.

Results

Dilution water for the two test series ranged in hardness from 180 to 210 mg/l (as $CaCO_3$), alkalinity from 156 to 182 mg/l (as $CaCO_3$), pH from 8.0 to 8.6, and dissolved oxygen from 8.4 to 9.3 mg/l.

Test concentrations for fish and Daphnia during the two series of tests ranged in hardness from 180 to 308 mg/l, alkalinity from 156 to 232 mg/l, pH from 7.1 to 9.0, dissolved oxygen from <1 to 9.3 mg/l and temperature from 22 to 27°C.

The high alkalinity and hardness values, the extreme pH and temperature values, and the low dissolved oxygen values all occurred in high wastewater concentrations.

The toxicity test results for the two series of tests are given in Tables 3 and 4. The data in Tables 3 and 4 show that influent and effluent toxicity varied and that for both test series toxicity was reduced both between the influent and secondary and between primary and secondary effluents for tests with all species. In general, the secondary effluents from both treatment systems A and B were not very toxic, they had LC50 or EC50 values of 50% or greater. The results, except for three tests, essentially show no difference in toxicity between paired influent samples (A and B), primary effluent samples (A and B), and secondary effluent samples (A and B) collected on the same date. Paired samples from the two treatment systems for the same date give toxicity test results which might be expected from duplicate samples collected from the same system. The data also show no significant difference in toxicity between influent and primary effluents collected on the same date. Control survival was excellent for the two test series with all fathead tests having >90% and rarely <100% survival and Daphnia tests having >84% survival.

Additionally, data for the fathead minnow and for the Daphnia show no significant difference between results for the two species for the same test sample or for similar samples between treatment system A and B collected the same date. Microtox test data, however, indicate greater toxicity for influent and primary effluent than that shown by the fathead minnow and Daphnia tests. The results for the toxicity tests for secondary effluents are essentially similar for all species tested. Since 50% was the highest test concentration in some of the early Microtox tests and since it was not toxic, the EC50 for those tests was greater than 50%.

Discussion

The alkalinity, hardness, pH, and dissolved oxygen (DO) values of the test concentrations varied considerably. The low dissolved oxygen levels associated with the high test concentrations of influent and primary effluent would be expected on the basis of the high BOD and COD of the mixed industrial and domestic waste which was the influent to the pilot treatment systems (Table 5). Although the low dissolved oxygen in wastewater concentrations above 10% probably added to the stress of the fish and Daphnia in the influent and primary effluent static tests, aeration of the samples to raise DO was not considered. It would have significantly modified the samples and would have constituted additional treatment of the samples. Furthermore, volatile toxicants, if present, would have been stripped by the amount of aeration required to maintain 60% saturation or greater dissolved oxygen levels in samples with such high BOD and COD. Changes in pH due to aeration might also have changed ammonia toxicity. Ammonia was potentially one of the major toxicants in the influent and primary effluent, as can be seen in the data for Table 5. Temperature varied more than desired during some of the tests. However, this would not invalidate the conclusions within a set of tests for the same date because conditions would have been similar.

Overall, the trend of the toxicity data indicated that the spike of volatile pollutants at the level dosed caused no added toxicity as seen in the lack of differences in toxicity between control and spiked influents and effluents. From data in the cited literature, U.S. Environmental Protection Agency[6], for 12 of the 16 compounds, the level of toxicant even for the combination of compounds (Water Quality Criteria, 1972)[7] would probably not be expected to cause acute toxic effects in fathead minnow or Daphnia tests. The Microtox assay data would also indicate no effect of the spike, but no literature is available to

suggest whether the spike of pollutants at the concentration added should affect the bacteria used for the test.

In terms of test sensitivity, the Microtox assays showed lower EC50 values for the influent and primary effluent tests than comparable fathead minnow and Daphnia tests. Some of this difference may be caused by the different diluent water used for the Microtox test and the fact that it measures sublethal effects, while the fathead minnow and the Daphnia acute test measure lethal effects.

Conclusions

A spike of 16 volatile priority pollutants, continuously added at 50 µg/l each, did not affect the acute toxicity of influent or effluents of an experimental, conventional activated sludge, pilot wastewater treatment system compared to a control system which received no addition of toxicants. The spike of volatile priority pollutants at the concentration added was apparently not high enough to significantly increase influent toxicity or affect treatment based on the toxicity of the effluent of the spiked system compared to the unspiked control.

There was not a significant reduction in toxicity between influent and primary effluent, although there was a significant reduction in toxicity between influent and secondary effluent and between primary and secondary effluents for both systems.

Fathead minnow and Daphnia toxicity tests results for the same samples of influent and primary effluent were not significantly different. Microtox™ test values for the same influent and effluent samples were, however, lower than the Fathead minnow and Daphnia values. Fathead minnow, Daphnia, and Microtox™ test values, however, were similar for the secondary effluents and indicated low or no toxicity.

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TABLE 1.[a] NOMINAL OPERATING CONDITIONS FOR THE A AND B SYSTEMS USED ON THE VOLATILE PRIORITY POLLUTANT PROJECT

I. Design Flow, Qd = 204.39 m^3/d (133L/min)

II. Primary Clarifiers -

Diameter	= 2.97 m
Weir Diameter	= 2.77 m
SWD	= 3.66 m
Surface Area	= 0.68 m^2
Surface Overflow Rate	= 27.99 m^3/m^2d

III. Aeration Basins -

L:W:D	= 5.34:3.05:3.66 m
Surface Area	= 16.33 m^2
Volume	= 59.76 m^3
Residence Time (Qd)	= 7.5 h

IV. Secondary Clarifiers -

Diameter	= 3.63 m
SWD	= 3.66 m
Surface Area	= 10.36 m^2
Surface Overflow Rate	= 18.41 m^3/m^2d

[a]Modified from Petrasek[1]

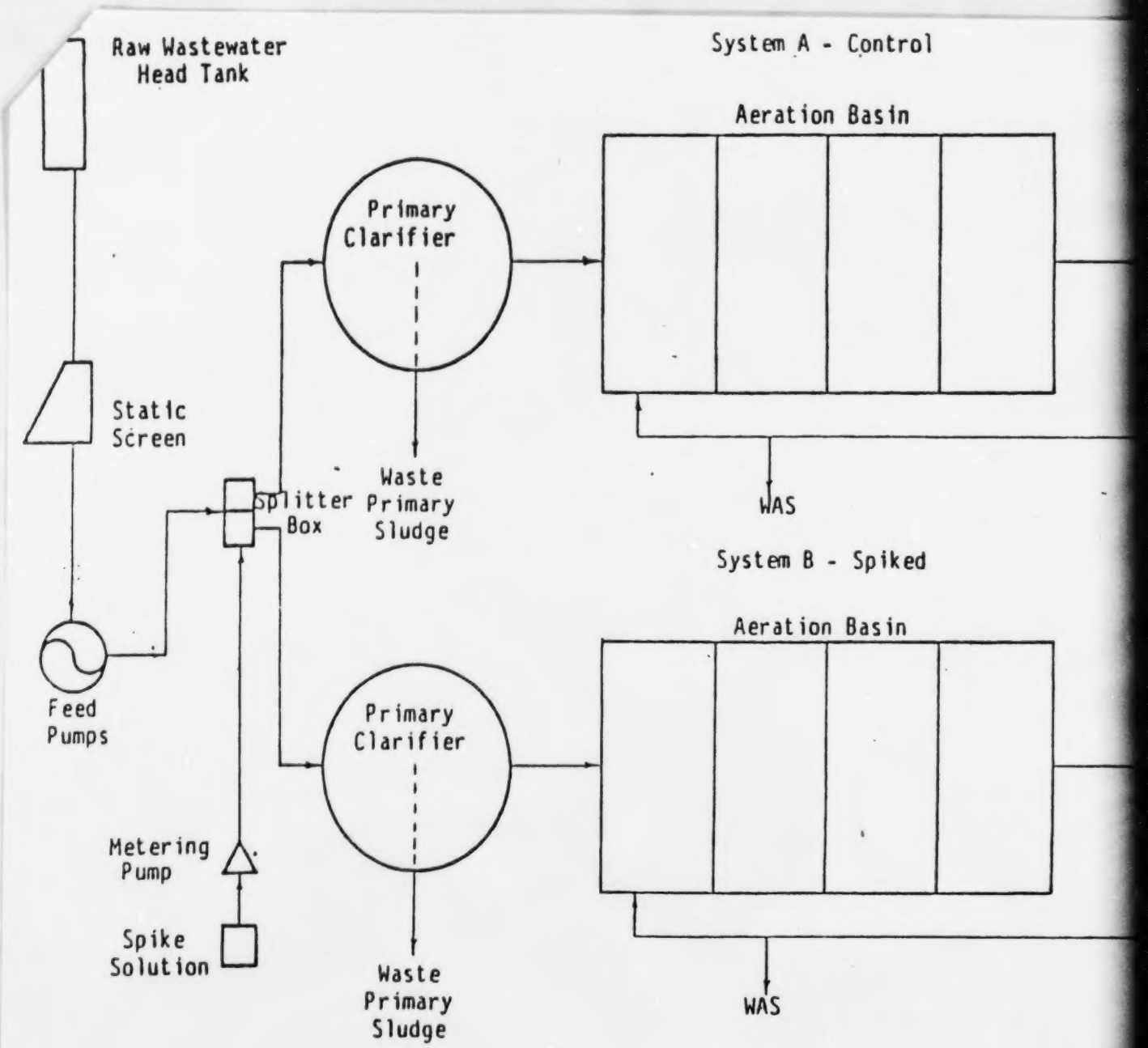

Figure 1.[a] Simplified Schematic Diagram of Systems

[a]From Petrasek[1]

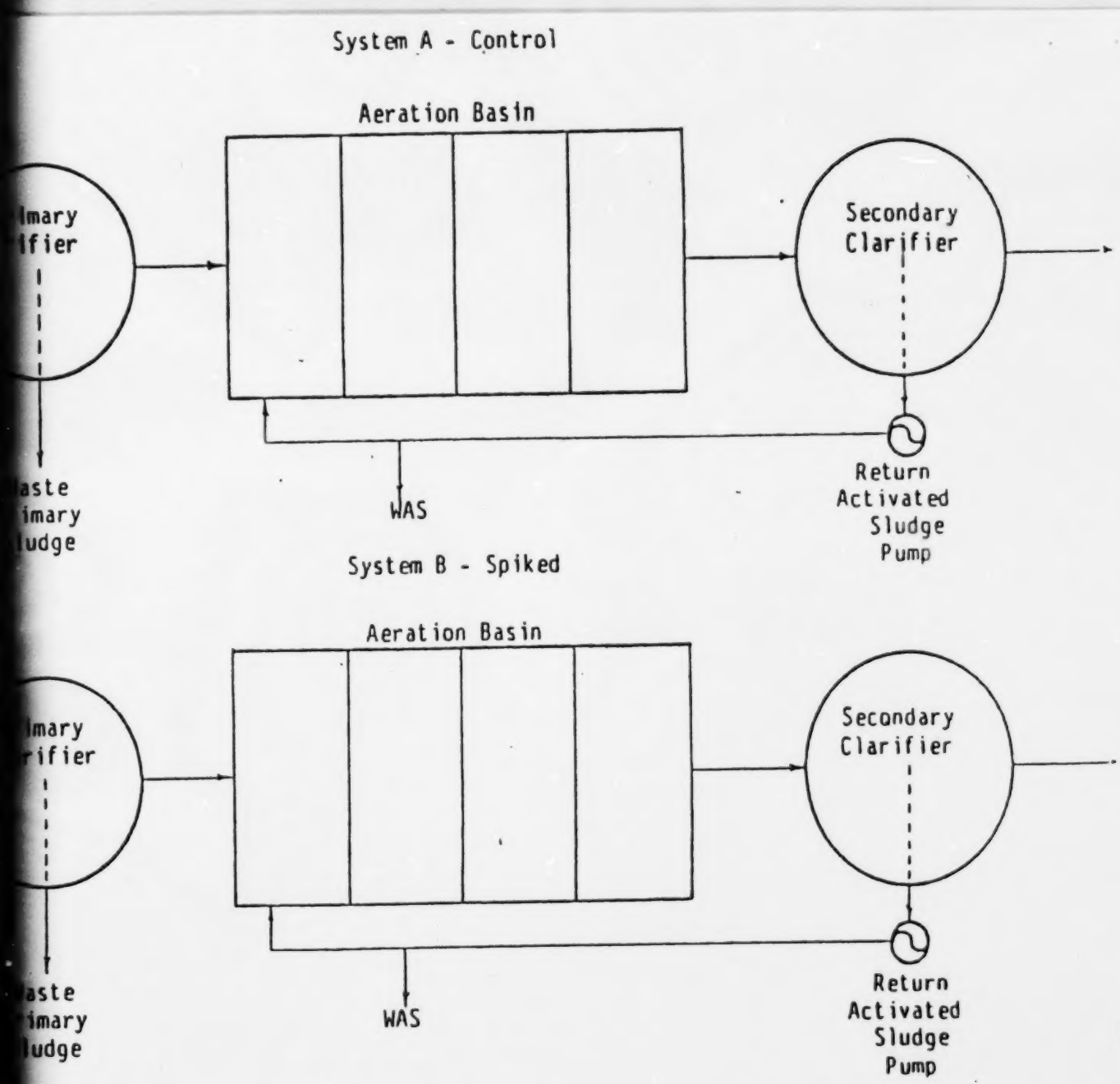

Figure 1.[a] Simplified Schematic Diagram of Systems A and B.

[a]From Petrasek[1]

TABLE 2. COMPOUNDS ADDED AND THEIR ACUTE TOXICITY VALUES

	Fathead Minnow 96-hr LC50 in µg/L[a]	Daphnia magna 48-hr EC50 in µg/L[a]
Methylene Chloride	310,000	224,000
1,1-Dichloroethene	169,000	11,600
Chloroform	-------[c]	28,900
Carbon Tetrachloride	43,100[b]	35,200
1,2-Dichloropropane	139,300[b]	52,500
Trichloroethylene	66,800	43,000
1,1,2-Trichloroethane	81,700[b]	18,000
Dibromochloromethane	-------	------
Benzene	32,000	203,000
1,1,1-Trichloroethane	105,000	-------
Bromodichloromethane	-------	-------
Chlorobenzene	29,120	86,000
Tetrachloroethylene	21,400	17,700
1,1,2,2,-Tetrachloroethane	20,300	9,320
Toluene	34,270	60,000
Ethylbenzene	42,330	75,000

[a]Static acute toxicity values, U.S. Environmental Protection Agency, 1980.
[b]Flow-through acute toxicity values, static values not available.
[c]No values available.

TABLE 3. FATHEAD MINNOW AND DAPHNIA MAGNA ACUTE TOXICITY VALUES FOR VPP INFLUENTS AND EFFLUENTS.

Date	Species	Influent LC50[a] and EC50[6] values in %		Secondary Effluent LC50 and EC50 Values in %	
		Control-A	Spiked-B	Control-A	Control-B
6/1/81	Fathead minnow	28.7 (34.7-24.5)	27.5 (33.6-23.1)	>100 (NC)[c]	>100 (NC)
	Daphnia magna	>50 (NC)	46.2 (73.4-36.3)	>100 (NC)	>100 (NC)
6/8/81	Fathead minnow	>50 (NC)	>50 (NC)	>50 (NC)	>50 (NC)
	Daphnia magna	NT --	NT --	>100 (NC)	>100 (NC)
6/15/81	Fathead minnow	48.3 (69.1-36.8)	50 (62.1-40.3)	>100 (NC)	>100 (NC)
	Daphnia magna	20.4 (27.0-15.9)	17.7 (22.8-13.7)	>100 (NC)	NT --
6/22/81	Fathead minnow	9.7 (13.2-7.4)	12.5 (16.4-8.6)	>100 (NC)	>100 (NC)
	Daphnia magna	9.8 (21.3-5.3)	11.4 (17.2-8.3)	>100 (NC)	>100 (NC)
6/29/81	Fathead minnow	17.7 (23.6-13.2)	8.8 (11.8-6.6)	>100 (NC)	>100 (NC)
	Daphnia magna	17.7 (21.3-14.7)	16.6 (20.1-13.6)	NA NA	>100 (NC)
7/6/81	Fathead minnow	9.7 (13.2-7.4)	9.7 (13.2-7.4)	>100 (NC)	>100 (NC)
	Daphnia magna	5.3 (6.3-3.3)	8.6 (10.4-7.0)	>100 (NC)	>100 (NC)
7/13/81	Fathead minnow	17.7 (23.6-13.2)	17.7 (23.6-13.2)	>100 (NC)	>100 (NC)
	Daphnia magna	20.9 (27.8-16.3)	20.0 (25.7-15.9)	>100 (NC)	>100 (NC)

TABLE 3. FATHEAD MINNOW AND *DAPHNIA MAGNA* ACUTE TOXICITY VALUES FOR VPP INFLUENTS AND EFFLUENTS (cont'd)

Date	Species	Influent LC50[a] and EC50[6] values in %		Secondary Effluent LC50 and EC50 Values in %	
		Control-A	Spiked-B	Control-A	Control-B
7/20/81	Fathead minnow	35.2 (49.2-28.7)	35.2 (49.2-28.7)	>100 (NC)	>100 (NC)
	Daphnia magna	41.9 (67.2-32.9)	39.8 (56.2-32.5)	>100 (NC)	>100 (NC)
7/27/81	Fathead minnow	25.0 (31.0-20.1)	28.7 (34.7-24.5)	>100 (NC)	>100 (NC)
	Daphnia magna	17.0 (22.9-12.5)	43.1 (99.8-29.7)	>100 (NC)	>100 (NC)

[a]Fathead minnow 96-hr LC50 and 95% confidence limits.

[b]*Daphnia magna* 48-hr EC50 and 95% confidence limits.

[c](NA)- test not acceptable. Excessive control mortality and/or mortality not concentration related.

[d](NC) - not calculable.

[e](NT) - not tested.

TABLE 4. FATHEAD MINNOW AND DAPHNIA MAGNA ACUTE TOXICITY VALUES AND MICROTOX BIOASSAY VALUES FOR VPP INFLUENTS AND EFFLUENTS.

Date	Species	Influent LC50[a] and EC50[b] values in %		Primary Effluent LC50 and EC50 values in %		Secondary Effluent LC50 and EC50 values in %	
		Control-A	Spiked-B	Control-A	Spiked-B	Control-A	Spiked-B
8-11-81	Fathead Minnow	23.6 (33.6-18.0)	22.3 (31.2-17.0)	28.7 (34.7-24.5)	25.0 (31.0-20.1)	>100 (NC)[c]	>100 (NC)
	Daphnia magna	15.9 (20.5-12.2)	14.2 (18.5-10.3)	NA[d] -	23.5 (29.5-19.4)	NT[e] -	NT -
	Microtox™	<6.3	<6.3	<6.3	<6.3	>50	>50
8/19/81	Fathead Minnow	26.8 (32.8-22.3)	20.9 (28.9-15.9)	23.6 (33.6-18.0)	25.0 (31.0-21.1)	>100 (NC)	>100 (NC)
	Daphnia magna	16.4 (28.8-8.5)	16.1 (22.2-11.2)	24.0 (34.5-18.3)	21.7 (31.9-15.9)	NT -	NT -
	Microtox™	4.3	5.6	<6.3	<6.3	>50	>50
8/25/81	Fathead Minnow	23.6 (33.6-18.0)	23.6 (33.6-18.0)	26.8 (32.8-22.3)	20.9 (28.9-15.9)	>100 (NC)	>100 (NC)
	Daphnia magna	31.6 (38.0-27.5)	40.5 (55.6-33.4)	34.7 (42.3-30.0)	35.6 (45.4-30.2)	>100 (NC)	>100 (NC)
	Microtox™	11.1	6.6	<6.3	<6.3	>50	>50
9/1/81	Fathead Minnow	38.7 (61.9-30.4)	>50 (NC)	>50 (NC)	>50 (NC)	>100 (NC)	>100 (NC)
	Daphnia magna	>50 (NC)	38.2 (51.7-31.6)	45.0 (83.7-34.6)	>50 (NC)	>100 (NC)	>100 (NC)
	Microtox™	19.5	21.9	5.2	4.3	>100	>100
9/7/81	Fathead Minnow	50.8 (62.9-41.2)	48.1 (73.9-35.2)	53.5 (65.7-44.6)	47.3 (67.3-36.1)	>100 (NC)	> 100 (NC)
	Daphnia magna	66.3 (78.6-58.1)	54.9 (76.1-44.9)	72.6 (91.2-62.1)	61.2 (74.2-52.7)	>100 (NC)	>100 (NC)
	Microtox™	13.6	12.8	23.9	16.5	>100	>100

TABLE 4. (CONTINUED) FATHEAD MINNOW AND DAPHNIA MAGNA ACUTE TOXICITY VALUES AND MICROTOX BIOASSAY VALUES FOR VPP INFLUENTS AND EFFLUENTS.

Date	Species	Influent LC50[a] and EC50[b] values in %		Primary Effluent LC50 and EC50 values in %		Secondary Effluent LC50 and EC50 values in %	
		Control-A	Spiked-B	Control-A	Spiked-B	Control-A	Spiked-B
9/14/81	Fathead Minnow	29.1 (39.3-21.7)	26.4 (34.7-18.6)	35.4 (47.2-26.5)	26.4 (34.7-18.6)	>100 (NC)	>100 (NC)
	Daphnia magna	22.3 (27.7-18.4)	22.3 (27.7-18.4)	22.9 (28.6-18.9)	21.8 (27.0-18.0)	NA	>100 (NC)
	Microtox™	7.2	6.3	6.3	<6.3	>100	>100

[a] Fathead minnow 96-hr. LC50 and 95% confidence limits.

[b] Daphnia magna 48-hr. EC50 and 95% confidence limits. Microtox 15-min. EC50 without confidence limits.

[c] (NA) - test not acceptable. Excessive control mortality and/or mortality not concentration related.

[d] (NC) - not calculable.

[e] (NT) - not tested.

TABLE 5.[a] PERFORMANCE SUMMARY OF VOLATILE PRIORITY POLLUTANT TREATMENT SEQUENCES; JANUARY-JUNE 1981

Parameter	Inf. (mg/l)	Pri. Eff. (mg/l)	Rem. by Pri. Clar. (%)	Activ. Sludge Eff. ------(mg/l)------		Overall Removal ---(percent)---	
				Control	Spike	Control	Spike
TSS	447.0	214.0	52.0	30.0	23.0	93.0	95.0
COD	577.0	317.0	45.0	91.0	87.0	84.0	85.0
Total-P	9.3	6.0	35.0	3.1	2.8	67.0	70.0
TKN	43.5	36.7	16.0	19.4	18.4	55.0	58.0
Organic N	20.4	14.2	30.0	5.7	5.2	72.0	75.0
NH_3-N	23.1	22.5	3.0	13.2	13.2	43.0	43.0
NO_2 & NO_3-N	0.2	0.2	-	6.4	6.3	-	-
Total-N	43.7	36.9	16.0	25.8	24.7	41.0	43.0
Turbidity (NTU)	-	-	-	12.0	10.0		
UCOD*	683.0	421.0	38.0	152.0	148.0	78.0	78.0

* UCOD = Ultimate Combined Oxygen Demand

= COD + 4.6 (NH_3-N)

[a] From Petrasek[1]